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Development And Evaluation Of Sustainable Mycelium-Based Packaging Material Using Agro-Fruit-Vegetable Waste And Mould Selection

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Abstract
Innovation in sustainable alternatives has been spurred by the escalating environmental crisis brought on by non-biodegradable plastic packaging. Using fungal mycelium cultivated on home fruit and vegetable waste (such as banana, potato, and mixed peels) and agricultural leftovers (such as wheat and rice straw), this study investigates the creation of biodegradable packaging materials. Thermally pasteurized substrate combinations were inoculated with a pure colony of fungi, molded, and then incubated under carefully monitored circumstances. Mycelial colonization, texture, binding strength, and the effect of various mold materials (silicone, HDPE, wood, resin, POP, jute, etc.) on demoulding ease were all evaluated in the study.
The findings showed that substrates that combined fibrous and starchy waste (such as potato peel and mixed fruit) resulted in dense, homogeneous mycelial development with excellent structural integrity. Because silicone and HDPE molds are flexible and non-stick, they allowed for excellent demolding. This study shows how kitchen and agricultural waste can be turned into useful, biodegradable packaging materials. The results back up the use of myco-packaging as a scalable, affordable, and environmentally beneficial substitute for traditional plastics, providing a workable answer for waste management and the circular bioeconomy.
***Keywords**:Mycelium-based packaging, agro-waste utilization, fruit and vegetable waste, fungal colonization, plastic alternative*

1.INTRODUCTION

The hunt for sustainable and biodegradable substitutes for synthetic packaging materials has accelerated due to the global concern over plastic litter (Balaeş, T. et. al., 2023). Traditional plastic packaging, which is mostly made of petrochemicals, is not biodegradable and greatly contributes to marine and terrestrial pollution, which poses serious problems for the environment and human health (Geyer et al., 2017). Due to its biodegradability, low energy consumption, and potential to replace fossil-based plastics, mycelium-based packaging, a type of biomaterial formed from the root-like structures of fungi has drawn a lot of attention among the developing solutions (Jones et al., 2020; Ghazvinian, A et. al., 2019).
On a range of lignocellulosic substrates, mycelium, the vegetative portion of fungi, can grow quickly and bind the material together during colonization. When the structure dries, it becomes biodegradable, strong, and lightweight (Holt et al., 2012). Because of this special quality, it is ideal for creating packaging elements including trays, containers, insulation boards, and protective inserts. The colonization rate, density, mechanical strength, and surface polish of the finished product are all significantly influenced by the mycelial growth medium (Appels et al., 2018). Agro-waste substrates, especially rice and wheat straw, are abundant in cellulose and hemicellulose, widely accessible, and frequently utilized as fungal growth media (Guan et al., 2017; Angelova, G. et. al., 2021; Haneef, M. et. al. 2017; Jones, M. et. al., 2018; Manan, S et. al., 2021). Furthermore, waste from fruits and vegetables, such as pea pods, banana peels, potato peels, and mango skins, are rich in sugars, starches, and other nutrients that improve biomass production and fungal metabolism (Mahmud et al., 2020). In addition to offering a rich nutrient base, the combination of kitchen waste and agricultural waste substrates solves the expanding problem of organic waste management in both urban and rural locations (Ardanuy, M. et. al., 2015).

Additionally, the quality and success of the demoulding process, as well as the texture and finish of the final product, are significantly impacted by the type of mold that is used to create the packaging material. The breathability, flexibility, and compatibility with fungal development of common mold materials differ, including HDPE plastic, silicone, wood, resin, and natural fibers. Designing an effective myco-packaging production system requires optimizing mold types based on fungal adherence, ease of removal, and reusability (Elsacker et al., 2021). Using a combination of domestic fruit and vegetable waste and agricultural leftovers, this study attempts to generate and examine mycelium-based packaging materials across a range of mold kinds. The study looks into the final packaging product's binding strength, mycelial texture, and fungal colonization behavior. The ultimate objective is to use locally accessible biodegradable resources to create a scalable, affordable, and biodegradable substitute for conventional packaging.

MATERIAL AND METHODS

Potato Dextrose Agar (PDA), a popular nutrient-rich medium for fungal growth, was employed to maintain a pure fungal culture of Ganoderma lucidum (Stamets, 2000). In accordance with techniques frequently used in the development of edible mushrooms, spawn was generated using sterilized brown rice as the carrier substrate and incubated at 25–28°C until fully colonized (Royse, 2003).

The goal of the current study was to create sustainable packaging materials based on mycelium by combining waste from fruits, vegetables, and agriculture. Local farms provided agricultural leftovers, such as rice and wheat straw, which are known to be high in lignocellulose and frequently utilized as fungal substrates (Guan et al., 2017). Banana, potato, mango, orange, watermelon, pomegranate, pineapple, pea, onion, leafy vegetable, and mixed vegetable peels were among the household fruit and vegetable wastes that were obtained from local markets and kitchens. It is known that these wastes, which are high in sugars, starches, and fibrous material, improve the metabolism of fungi (Mahmud et al., 2020).

All gathered materials were air-dried, partially crushed to promote fungal penetration and nutrient availability, uniformly chopped to guarantee surface area for colonization, and carefully cleaned with distilled water to remove dirt and pesticide residues (Jones et al., 2020). Agricultural waste with vegetable waste, agricultural waste with fruit waste, and agricultural waste with mixed fruit and vegetable waste were the three experimental categories into which the substrates were divided. The substrates were thermally pasteurized for microbial decontamination by heating them to 80–90°C for 45 minutes. This process has been shown to be successful in lowering competitive bacteria while maintaining substrate quality (Zervakis et al., 2001; Kupradi, C et. al. 2017).Following aseptic chilling, distilled water was added to each mixture to maintain an ideal moisture level of 60–70%, which is essential for hyphal growth and fungal enzymatic activity (Appels et al., 2018). 10% (w/w) of fungal spawn was added to the pre-treated substrates, mixed well, and then put into sterile molds. Following that, the inoculated substrates were incubated at 25–28°C in a dark environment with a relative humidity of 70–80%, which has been found to be optimal for quick and consistent mycelial colonization in earlier research (Elsacker et al., 2021). Monitoring was done every day to track the colonization process, find contaminants, and evaluate the surface mycelium's texture.Following full colonization, the old mycelium-substrate blocks were meticulously demolded and dried in an oven set to 40–50°C for 12–24 hours. By solidifying the structure and inactivating the active mycelium, this technique produces a stable, biodegradable packing material (Holt et al., 2012). Colonization time, mycelial texture (such as dense, fluffy, fibrous, or spotty), binding strength (manually assessed as low, medium, or high), and ease of demoulding were the criteria used to evaluate the dried packaging units.The best-performing substrate combination (based on fungal colonization and material quality) was chosen and filled into a range of mold types, including plastic (HDPE), silicone, plywood (wood), aluminum (metal), cardboard, epoxy resin, jute fiber, and plaster of Paris (POP), in order to assess the most appropriate mold type. The form retention and ease of demoulding of mycelial products are directly impacted by the porosity, flexibility, and surface adhesion of these materials (Jones et al., 2020; Elsacker et al., 2021). The molds were meticulously demolded and dried in an oven and each sample was then assessed for material integrity and demolded effectiveness (Teeraphantuvat, T. et. al., 2024).

RESULT AND DISCUSSION

Preparation Of Packaging Material With Different Vegetable Waste Substrate Along With Agricultural Waste

S.No	Substrate Taken	Texture of Mycelium	Binding Strength
1	Wheat straw (<1 cm) + Rice straw (<1 cm) + Potato peel	Dense and even	High
2	Wheat straw (<1 cm) + Rice straw (<1 cm) + Onion peel	Thin, patchy	Low-Medium
3	Wheat straw (<1 cm) + Rice straw (<1 cm) + Pea peel	Compact and fibrous	Medium-High
4	Wheat straw (<1 cm) + Rice straw (<1 cm) + Mixed vegetable peel	Fluffy, full coverage	Medium
5	Wheat straw (<1 cm) + Rice straw (<1 cm) + Leafy vegetable waste	Smooth but loose	Medium-Low

Table- Table showing results of packaging material with different vegetable waste substrate along with agricultural waste

Depending on the kind of vegetable waste utilized, mycelium-based packaging materials exhibit a wide range of growth and structural performance. Due to the high starch content that promotes fungal adherence, the combination of potato peel, rice straw, and wheat straw produced dense, uniform mycelial growth with a high binding strength. Because of its antibacterial properties, onion peel produced low to medium strength and thin, spotty growth. Pea peel, perhaps because of its well-balanced nutritional makeup, promoted compact, fibrous growth with medium-high binding. The uneven substrate composition was reflected in the fluffy, soft, and somewhat strong mycelium produced by mixed vegetable peels. Due to poor fiber and probably too much moisture, leafy vegetable waste generated smooth but loose mycelium with medium-low binding. Both mechanical stability and colonization were improved by starchy and fibrous substrates.

Preparation Of Packaging Material With Different Fruit Waste Substrate Along With Agricultural Waste

S.No	Substrate Taken	Texture of Mycelium	Binding Strength
1	Wheat straw (<1 cm) + Rice straw (<1 cm) + Banana Peel	Dense and fluffy	High
2	Wheat straw (<1 cm) + Rice straw (<1 cm) + Orange Peel	Thin, slightly brittle	Medium
3	Wheat straw (<1 cm) + Rice straw (<1 cm) + Pomegranate Rind	Patchy and uneven	Low
4	Wheat straw (<1 cm) + Rice straw (<1 cm) + Mango Peel	Thick and compact	Medium-High
5	Wheat straw (<1 cm) + Rice straw (<1 cm) + Watermelon Rind	Watery, inconsistent	Low
6	Wheat straw (<1 cm) + Rice straw (<1 cm) + Pineapple Peel	Thin, fibrous	Low-Medium
7	Wheat straw (<1 cm) + Rice straw (<1 cm) + Mixed Fruit Waste	Fluffy and uniform	High

Table- Table showing results of packaging material with different fruit waste substrate along with agricultural waste

The composition of the substrate has a significant impact on the mechanical integrity and physical properties of mycelium composites. Because of its high moisture and carbohydrate content, which promote quick hyphal development and solid binding, banana peel produced the best results among the studied combinations, exhibiting dense and fluffy mycelial growth and strong binding strength. The medium-strength, thin, and slightly brittle mycelium found in orange peel was probably caused by the essential oils and acidic pH, which could prevent regular colonization. Because of its rough texture and antibacterial phenolics that prevent fungal spread, pomegranate rind produced spotty, uneven growth with poor strength.Mango peel is a good substrate since it produces thick, compact mycelium with medium-high binding strength and is noted for having moderate levels of sugar and fiber. However, because of substrate instability and possible microbial competition, watermelon rind, which is low in structure and very watery, resulted in uneven development and poor binding. The fibrous yet acidic characteristic of pineapple peel resulted in low to medium strength and thin mycelial layers, suggesting limited applicability. Last but not least, one of the most promising substrates for the production of myco-composite was mixed fruit waste, which provides a balance of nutrients and moisture and encouraged fluffy and uniform development with high binding strength.

Preparation Of Packaging Material With Different Fruit And Vegetable Waste Substrate Along With Agricultural Waste

S.No	Substrate Taken	Texture of Mycelium	Binding Strength
1	Wheat straw (<1 cm) + Rice straw (<1 cm) + Banana Peel + Potato peel	Dense & uniform	Very High
2	Wheat straw (<1 cm) + Rice straw (<1 cm) + Mango Peel + Potato peel	Fluffy & compact	High
3	Wheat straw (<1 cm) + Rice straw (<1 cm) + Mixed Fruit Waste + Potato peel	Thick & uniform	Very High
4	Wheat straw (<1 cm) + Rice straw (<1 cm) + Banana Peel + Pea Peel	Fibrous & patchy	Medium
5	Wheat straw (<1 cm) + Rice straw (<1 cm) + Mango Peel + Pea Peel	Irregular, loose	Low-Medium
6	Wheat straw (<1 cm) + Rice straw (<1 cm) + Mixed Fruit Waste + Pea Peel	Smooth & compact	High
7	Wheat straw (<1 cm) + Rice straw (<1 cm) + Banana Peel + Mixed vegetable peel	Fluffy & firm	High
8	Wheat straw (<1 cm) + Rice straw (<1 cm) + Mango Peel + Mixed vegetable peel	Soft & patchy	Medium
9	Wheat straw (<1 cm) + Rice straw (<1 cm) + Mixed Fruit Waste + Mixed vegetable peel	Dense & robust	Very High

Table- Table showing results of packaging material with different fruit and vegetable waste substrate along with agricultural waste

Fruit and vegetable peels combined with agricultural waste as a substrate had varying impacts on the binding strength and mycelial development. Because of the high starch, sugar, and fiber content that promotes fungal colonization and structural integrity, the banana peel + potato peel and mixed fruit waste + potato peel mixtures produced dense, uniform growth with very high binding strength. Additionally, compact development with high binding was encouraged by mango and potato peels.

Pea peel combinations (e.g., with banana or mango) exhibited medium to low strength and patchy or loose growth, indicating insufficient nutrient synergy. Mixed vegetable peel substrates supported strong,

robust mycelial textures with high to very high strength, and they functioned better when combined with banana peel or mixed fruit waste. Mixed fruit + mixed vegetable peel was the most successful substrate, suggesting that a variety of organic content improves composite strength and colonization.
Packaging Material With Different Mould Type

S.No	Mould Type	Material	Ease of Demoulding
1	Plastic Mould (HDPE)	Plastic	High
2	Silicone Mould	Silicone Rubber	Very High
3	Wooden Mould (Plywood)	Wood	Moderate
4	Metal Mould (Aluminium)	Metal	Low
5	Cardboard Mould	Paperboard	Low
7	POP Mould	Plaster of Paris	Low
8	Natural Fiber (Jute)	Organic Fiber	Low
9	Resin Mould (Epoxy)	Synthetic Resin	High

Table- Table showing results of packaging material with different mould types
The ease with which mycelium-based packaging can be demoulded during colonization and drying is largely dependent by the mold material. The efficiency of several molds with varying surface characteristics and material compositions was evaluated in this study. Because silicone molds are naturally non-stick, flexible, and hydrophobic, they demonstrated the greatest ease of demoulding. These characteristics enable the composite to be released easily without causing structural damage. Similarly, due to their smooth surfaces and little adherence to the mycelial matrix, HDPE plastic molds also showed high demoulding efficiency.Although they are not as flexible as silicone, resin molds (epoxy) offer smooth surfaces and structural stiffness that allow for clean release. They also have a high demoulding capacity. Because of their rough texture and surface porosity, wooden molds (plywood) were moderately easy to use but occasionally stuck, necessitating cautious handling to prevent breakage. Demoulding was difficult with cardboard and aluminium metal molds. Cardboard absorbs moisture and causes warping and adhesion, whereas metal surfaces have a tendency to stick to the damp mycelial substance. Because POP and jute fiber molds are both highly absorbent and uneven, the mycelium adhered tightly to them and broke when they were removed, which is why they also performed badly.

CONCLUSION

Using combinations of agricultural, fruit, and vegetable waste substrates molded in various materials, the current study effectively illustrated the creation of sustainable mycelium-based packaging materials. The results show that mycelial colonization, texture, and the quality of the final package are all greatly influenced by the composition of the substrate. The combination of potato peel, rice straw, and wheat straw produced thick, uniform mycelial growth with a high binding strength, making it the most appropriate of the evaluated vegetable wastes among the vegetable-based substrates. Because of their rich carbohydrate and moisture profiles, banana peel and mixed fruit waste were shown to be the most efficient fruit-based additions, generating dense, fluffy, and homogeneous mycelium with high to very high mechanical strength. Fruit and vegetable peels were blended with agricultural leftovers to improve colonization and structural quality. This was especially true for substrates like banana + potato peel and mixed fruit + mixed vegetable peel, which showed strong mycelial networks and very high binding strength. These results confirm that proper mycelial development is supported by a synergistic nutritional profile derived from various organic wastes. The study also found that the type of mold has a significant impact on how easy it is to demould. The best demoulding results were obtained with silicone and HDPE plastic molds because of their flexibility and non-stick surfaces. On the other hand, poor performance was demonstrated by cardboard, POP, jute, and metal molds, which frequently resulted in substrate adhesion and breaking after removal. Using nutrient-rich, starch- and fiber-containing agro-waste and organic peels, mycelium-based packaging can be made efficiently. The choice of mold type is crucial for shaping and releasing the finished product. These results provide credence to the viability of environmentally friendly, biodegradable packaging substitutes that may eventually take the place of traditional plastic packaging.

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